CLAIMS

1. A multilayer enzyme immobilization process comprising: adsorbing a polyethyleneimine solution onto a fibrous matrix, said matrix including a plurality of fibrils;

adding an enzyme in solution to said fibrous matrix;

forming at least two layers of polyethyleneimine-enzyme aggregates on said fibrils; and

cross-linking said polyethyleneimine-enzyme aggregates.

- 2. The process of claim 1 wherein said enzyme is selected from β -galactosidase, lipase, lactate dehydrogenase, formate dehydrogenase, glucose isomerase, and combinations thereof.
- 3. The process of claim 2 wherein said β -galactosidase is isolated from a microorganism selected from B. circulans, B. singularis, T. aquaticus, K. lactis, E. coli, A. oryzae, A. niger, or combinations thereof.
- 4. The process of claim 3 wherein said β -galactosidase is isolated from the microorganism A. oryzae, B. circulans, K. lactis, or combinations thereof.
- **5**. ` The process of claim 1 wherein said fibrous matrix is selected from cotton fibers, poly(ethylene terephthalate), glass fiber, wool, carbon fiber, ceramic fiber, paper, rayon, or combinations thereof.
- 6. The process of claim 1 wherein said fibrous matrix comprises an enzyme load that is less than about 500 mg/g fibrous matrix after adding said enzyme solution.
- 7. The process of claim 1 wherein said polyethyleneimine solution comprises polyethyleneimine solubilized in water.

- 8. The process of claim 7 wherein the concentration of polyethyleneimine in said polyethyleneimine solution is between about 0.001 mg/mL and the solubility of polyethyleneimine in water.
- 9. The process of claim 7 wherein the concentration of polyethyleneimine in said polyethyleneimine solution is between about 0.001 and about 30 mg/mL.
- 10. The process of claim 7 wherein the concentration of polyethyleneimine in said polyethyleneimine solution is about 2 mg/mL.
- 11. The process of claim 1 wherein said enzyme solution comprises enzyme solubilized in water.
- 12. The process of claim 11 wherein the concentration of enzyme in said enzyme solution is between about 0.001 and about 100 mg/mL.
- 13. The process of claim 1 wherein said polyethyleneimine molecules are positively charged, said enzyme is negatively charged, and said fibrous matrix comprises a balanced charge ratio of said polyethyleneimine to said enzyme.
- 14. The process of claim 1 wherein said fibrous matrix comprises a ratio of polyethyleneimine to enzyme of between about 1/33.3 and about 1/8.3 after adsorbing said polyethyleneimine solution in said fibrous matrix and adding said enzyme solution.

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15. The process of claim 14 wherein said fibrous matrix comprises a polyethyleneimine to enzyme ratio of between about 1/20 and about 1/25 after adsorbing said polyethyleneimine solution in said fibrous matrix and adding said enzyme solution.

- 16. The process of claim 15 wherein said fibrous matrix comprises a polyethyleneimine to enzyme ratio of between about 1/22 and about 1/25.
- 17. The process of claim 1 further comprising maintaining said polyethyleneimine and enzme solution at a pH in the range of between about 4 and about 10 during said forming at least two layers of polyethyleneimine-enzyme aggregates.
- 18. The process of claim 17 wherein said pH is between about 6 and about 8.
- 19. The process of claim 1 further comprising maintaining said enzyme solution at a temperature that is less than about 65°C prior to adding to said fibrous matrix.
- 20. The process of claim 19 further comprising maintaining said enzyme solution at a temperature that is between about 0 and about 25°C prior to adding to said fibrous matrix.
- 21. The process of claim 1 further comprising forming at least two layers of polyethyleneimine-enzyme aggregates on said fibrils at a temperature that is less than about 65°C.
- 22. The process of claim 1 further comprising forming at least two layers of polyethyleneimine-enzyme aggregates on said fibrils at a temperature that is between about 0°C and room temperature.
- 23. The process of claim 1 further comprising forming at least two layers of polyethyleneimine-enzyme aggregates on said fibrils such that said aggregates completely cover said fibrils.
- 24. The process of claim 1 wherein said cross-linking of said polyethyleneimineenzyme aggregates is performed by applying an enzyme fixative.

- 25. The process of claim 24 wherein said enzyme fixative is an aldehyde or keto compound that can form covalent bonds with the amine groups of an enzyme protein.
- 26. The process of claim 24 wherein said enzyme fixative is gluteraldehyde, formaldehyde, or combinations thereof.
- 27. The process of claim 26 wherein said gluteraldehyde comprises a solution of gluteraldehyde and water.
- 28. The process of claim 27 wherein said gluteraldehyde solution has a concentration between about 0.05 and about 0.2%.
- 29. The process of claim 28 wherein said gluteraldehyde solution has a concentration of about 0.1%.
- 30. The process of claim 24 wherein said enzyme fixative has a pH between about 6 and about 8.
- 31. The process of claim 24 wherein said enzyme fixative is reacted with said polyethyleneimine-enzyme aggregates for a time sufficient to cross-link said polyethyleneimine-enzyme aggregates.
- 32. The process of claim 31 wherein said enzyme fixative is reacted with said polyethyleneimine-enzyme aggregates for at least about 5 minutes.
- 33. The process of claim 24 further comprising maintaining said enzyme fixative at a temperature that is less than about 65°C prior to cross-linking said polyethyleneimine-enzyme aggregates.

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34. The process of claim 1 further comprising washing said fibrils and said cross-linked polyethyleneimine-enzyme aggregates formed thereon with distilled water and acidic buffer subsequent to said cross-linking.

- 35. The process of claim 1 wherein said process produces an immobilized enzyme yield that is less than or equal to about 100%
- 36. The process of claim 1 wherein said process produces an immobilized enzyme yield that is at least about 5%.
- 37. A process of using multilayer immobilized enzyme in the production of galactooligosaccharides from lactose, said multilayer immobilized enzyme being prepared by the process of claim 1.
- 38. A process of using multilayer immobilized enzyme in the hydrolysis of lactose to glucose and galactose, said multilayer immobilized enzyme being prepared by the process of claim 1.
- 39. A fibrous-bed biocatalytic reactor for production of galacto-oligosaccharides from lactose, said reactor comprising:
 - a fibrous matrix disposed in a vessel, wherein

said fibrous matrix comprises said multilayer immobilized polyethyleneimine-enzyme aggregates prepared by the process of claim 1, and

said vessel is configured for the steady flow of a lactose solution.

- 40. A fibrous-bed biocatalytic reactor for the hydrolysis of lactose to glucose and galactose, said reactor comprising:
 - a fibrous matrix disposed in a vessel, wherein

said fibrous matrix comprises said multilayer immobilized polyethyleneimine-enzyme aggregates prepared by the process of claim 1, and

said vessel is configured for the steady flow of a lactose solution.